class rather than the pale class. Because of the very sharp expression of variegation among the Spm carrying kernels and also because of the faint pigment produced in its absence, this 5718 state of a_1^{m-1} has been useful in a number of studies of the Spm system.

Subsequent tests conducted with plants having state 5718 a_1^{m-1} and Spm have indicated that the appearance of kernels having the A_1 phenotype in its progeny is rare. The number with this phenotype derived from tests of plant 6046B-4 entered in table 15, is unusually high. ~~In this cases~~, The same collection of pollen was used ~~to make both the~~ for the self-pollination and for the test cross entered in this table. It is suspected that the tassel of this plant from which pollen was collected had a small mutant A_1 sector, and that the pollen grains carried the A_1 mutant and derived from anthers in this sector were responsible for the appearance of the ~~relatively~~ unusually high ~~large~~ number of kernels exhibiting the A_1 phenotype.

A number of plants were grown from kernels ~~derived~~ on the ears produced by crosses conducted with plant 5720. With rare exceptions, the state of a_1^{m-1} in this plant and in its progeny gives rise in plant and kernel to mutant areas that exhibit only low levels of pigment intensity. As mutation inducting events may occur very early in development of ~~either~~ the plant ~~tissues~~ and including ~~sporogenous~~ cells, ~~or in gametophytic cells, and in the kernel~~, a number of

gametes are formed by plants having this state that carry a mutant of a_1^{m-1}

Therefore, many kernels appear on test cross ears that express this.

One grade of pigment intensity is expressed in each but the range of this

among the mutant carrying kernels is from very faint to medium dark. (Photos - -)

When a plant having this state 5720 of a_1^{m-1} and also Spm is used as a

pollen parent in the cross to plants homozygous for a_1, only one of the

three chromosomes 3 in the endosperms of kernels on the resulting ear carries

this a_1^{m-1}. Among the variegated class of kernels with this constitution,

mutant areas appear. Each expresses one particular grade of pigment

intensity within it. When, however, the reciprocal cross is made, among

the variegated kernels on the resulting ear, such mutant areas but often appear within

~~which~~ from there are smaller areas exhibiting deeper shades of pigment intensity.

This is expected because in these kernels ~~have this state of~~ state 5720 a_1^{m-1} is carried in two of its

three chromosomes 3, ~~and~~ mutation-inducing events occur to each independently

of ~~one another~~. Types of kernels appearing one ears produced ~~from~~ by the cross ~~of~~ would give rise to this type of variegated expression.

of ~~some~~ plants that were a_1^{m-1} (state 5720)/a_1 to plants homozygous for a_1

are given in table 16. In this table it will be seen that the number of

uniformly pigmented kernels is high as ~~well as~~ so the number of colorless

kernels. It was subsequently learned that with state 5720, no anthocyanin Among the uniformly pigmented class of kernels, not one but many different grades of pigment intensities were represented, each kernel showing one particular grade of them.

pigment is produced in either plant or kernel when Spm is absent. ~~There~~

The high rate of germinal mutation produced by this 5720 state of a_1^{m-1} in the presence of Spm resembled that produced by the original state of a_1^{m-1} from which it was derived. However, with this original state, the majority of germinal mutations give rise to ~~the full or near full A_1 type expression~~ mutants that express high levels of pigment intensity resembling or approaching that produced by A_1. ~~The proportion of germinal mutations~~ Those that express ~~lower~~ levels of pigment intensity ~~to a considerably lesser~~ much lower frequencies.

Because of the very high rate of germinal mutation given by the original state of a_1^{m-1}, ~~it~~ this state was not considered to be a useful one for analysis of the mode of operation of the Spm system and tests of it were not continued beyond the 1951 season. In contrast, state 5720, which is similar to the original state of a_1^{m-1} in regard to time of occurrence of mutation but not in regard to 5720, has been quite useful.

5. Individuality of expression of each state of a_1^{m-1} when two different ones are ~~combined~~ present in a plant or kernel.

During the summer of 1951, crosses were made between plants carrying different states of a_1^{m-1} and the type of expression ~~of~~ given by each state ~~in~~ among the kernels on the resulting ear was examined. ~~The Combinations of states were as follows:~~ Crosses were made that would bring together in a single kernel the following states of a_1^{m-1}: state 5719A-1 with state 5720 and with the original state, state 5718 with the original state, state 5720 with the original state, and state 5719A-2 with state 5700A, not previously discussed. In later years, other combinations of states were made. However, no additional [illegible] information of general interest was gained from study of plants having these.

The most instructive cross ~~of that~~ illustrated the individuality of each state~~s~~ of a_1^{m-1} when combined in a kernel or plant was that (made) between a plant~~s~~ carrying state 5719A-1 ~~to~~ and one ~~plants~~ carrying state 5720, ~~and~~ The types of kernels appearing on ~~the~~ an ear produced by one such cross will be given. Pollen from plant 6042-9, that carried state 5720, was placed on the silks of a tiller ear of plant 6046C-1 which ~~carried state 5719A-1~~ was homozygous for state 5719A-1. In order to make clear the results obtained from this cross, it will be necessary to consider other test crosses made with each of these two plants.

¶ The first ear of the main stalk of plant 6046C-1 was self-pollinated and on the resulting ear there were 4 uniformly pigmented kernels exhibiting ~~the~~ each A_1-type pigment, 31 kernels that were uniformly ~~pale colored~~ pigmented, but the intensity of this was low, and it was the same in all kernels in this class, ~~and~~ 377 kernels ~~that~~ had small spots of the A_1 type pigment in a colorless background,-- the typical pattern produced by state 5719A-1. In addition there were and 1 kernel that appeared to be totally colorless. Since plant 6046C- was [illegible] in constitution, the classification of kernel types was readily accomplished. Pollen of this plant was ~~also~~ placed on the silks of a plant homozygous for a_1 and on the resulting ear there was 1 kernel exhibiting the A_1 phenotype, ~~uniformly pigmented kernels showing the of the A_1 type~~,

212 kernels that were uniformly pale colored, and 465 that expressed the variegation pattern typically produced by state 5719A-1. Plant 6042-9 had been derived from a variegated kernel on the self-pollinated ear of plant 5720. Pollen from a plant homozygous for a_1 had been placed on the silks of a tiller ear of this plant. The kernel types on this ear were as follows: 32 uniformly pigmented kernels, each showing a particular level of pigment intensity in the aleurone layer but in all of them this level was low, 102 variegated kernels with large and medium and small areas exhibiting low levels of pigment intensity in them and a colorless background, and 239 kernels in which no pigment was observed. When pollen of plant 6042-9, with state 5720, was placed on silks of an ear of a tiller of plant 6046C-1, it was learned that the pollen parent was homozygous for state 5720. Thus, the cross was made between a plant homozygous for state 5719A-1 and one homozygous for state 5720. There were 216 kernels on the ear this cross produced. Four of them were uniformly pale colored. In 18 others, spots with A_1-type pigment were present in a lightly pigmented background and the pattern of the A_1 spots was that characteristically produced by state 5719A-1. The intensity of the background pigment was uniform over the aleurone layer in each kernel but the level of this differed among these kernels. And, the ranges of these levels were the same as that

appearing among the uniformly pigmented kernels on ears produced by the cross of plants having state 5720 to plants homozygous for a_1. In addition, there were 194 variegated kernels of another type. These kernels had the pattern of A_1 spots produced by state 5719A-1 and also the pattern of lightly pigmented areas characteristically produced by state 5720 but the background was colorless (see photos). The over-all pattern was that which could be expected from superposition of the pattern produced by one state over that produced by the other would arise on that produced by the other. Each state expressed its own type of response to the presence of Spm.

It was assumed that in the 18 kernels showing the variegation pattern produced by state 5719A-1 on a uniformly pigmented background, ~~a mutant~~ instead of a colorless one, had been produced by fertilization of a male gamete carrying a mutant derived from state 5720, and that this ~~mutant~~ mutant was stable in the presence of Spm, ~~had been contributed by the parent carrying state 5720.~~ The correctness of this interpretation was made evident in tests of the plants derived from kernels of this type that were conducted in ~~a~~ subsequent years. The plant derived from each carried a mutant of a_1^{m-1} in one chromosome 3, whose expression was stable either in the presence or the absence of Spm, and the state 5719A-1 of a_1^{m-1} in the homologue, which continued to express its individual variegation pattern in subsequent generations.

A ~~description~~ discussion of the types of test that established this will be postponed until a description may be given of the means by which such tests are made possible. On contrast, those plants arising from the 2nd class of variegated kernels carried state 5720 as a_1^{m-1} in one chromosome 3 and state 5719A-1 in the homologue. Both states indicated from these plants neither appeared to have been affected in any way by the presence of Spm.

As described earlier (page.), plant 5700A was derived from a very pale colored kernel on an ear produced by cross of the original a_1^{m-1} carrying plant to one that was homozygous for a_1. Pollen of plant 5700A was placed on silks of an ear of a plant homozygous for a_1. Two types of kernels appeared on this ear, those that were very pale in color and those that were colorless. An accurate classification of the two types was not attempted as the intensity of pigment in the pale class was so low that it was feared that some belonging in the class would fail to be detected and would be placed with the colorless class. Some kernels that certainly had pigment in them were selected from this ear, and the plants grown from them in the summer of 1951 were given culture number 6078. At least one ear of each plant in this culture received pollen from a plant homozygous for a_1 and sh_2. On all ears so produced, there were only the two classes of kernels; very pale colored and colorless, ~~and~~ an accurate ~~for each type~~ distinction of type for all kernels ~~classifications~~ was also difficult to make. On some of these ears. As an example, silks of the first ear on the main stalk of plant 6078-5 received pollen from a plant homozygous for a_1 and sh_2. On the resulting ear, 256 kernels certainly had pigment in them, but the intensity was exceedingly low; ~~but~~ in the remaining 314 kernels pigment could not be detected by visual examination ~~appeared to be colorless~~. A tiller ear of this plant was self-pollinated and similar difficulties were encountered in making accurate classifications of the two classes of kernels that appeared on it. Among the 424 kernels

this ear produced, 254 had faint pigment in them but the remai ing 170 appeared to be colorless. The silks of an ear on another tiller of ~~this~~ 6078-5 plant received pollen from plant 6080C-7 whose constitution ~~was~~ was a_1^{m-1} ~~(state 5719A-2)/a_1~~ (~~For description of this state, see pages~~). The first ear on the main stalk of plant 6080C-7 ~~had been~~ was self-pollinated and among the 234 kernels ~~it produced~~ on this ear, one was fully A_1 in phenotype, none were pale, 172 were variegated for dots of A_1 in a colorless background, and 61 were colorless. The second ear on the main stalk received pollen from a plant homozygous for a_1. On the ear this cross produced there were 11 pale colored kernels, 97 showing dots of A_1 in a colorless background, and 127 that were colorless. Pollen collected from a tiller of ~~this~~ plant 6080C-7 was placed on the silks of an ear ~~produced by a second~~ of a tiller of plant 6078-5. The types of kernels appearing on this ear were surprising. There were 2 kernels exhibiting ~~the~~ full A_1-type pigmentation, 75 kernels that were uniformly pale colored, and 132 that appeared to be colorless.

In addition, there were 259 variegated kernels. Eighty-three of them had dots of A_1 in a colorless background--the pattern of variegation associated with state 5719A-2. The remaining 176 variegated kernels exhibited ~~a few large~~ areas ~~of~~ full A_1-type pigment ~~and also very many small spots of this that were situated quite close to one another~~. In some of these

kernels there were a few ~~pale~~ areas and in other a few areas that appeared to be colorless. It was suspected immediately that the plants in culture 6078 had a modified state of a_1^{m-1} but no Spm. Introduction of Spm through a male gamete of plant 6080C-7 revealed the pattern of variegation this state would produce in its presence. Subsequent tests substantiated this. They commenced with plants derived from the pale class of kernels on ears of ~~the~~ plants in culture 6078 that were produced by the cross with plants homozygous for a_1 and sh_2. The types of test conducted with these plants will be discussed later. ~~Both they and their progeny carrying this 5700A state of a_1^{m-1}~~ proved to be useful in examining some aspectes of the Spm system of opea tion. Plants derived from the newly appearing variegated class of kernels on the ear produced by the cross of 6078-5 by 6080C-7 were also examined. Some of them were a_1^{m-1}(state 5700A)/ a_1 and others were a_1^{m-1}(state 5700A)/a_1^{m-1} (state 5719A-2). ~~Both~~ states appeared in the progeny of plants having this latter constitution, each having retained its own properties while together in the nuclei of ~~one~~ plant. Discussion of the manner in which these tests were made will be postponed until the methods adopted for them may be considered.

Besides the intercrosses of plants carrying the distinctive states of a_1^{m-1}, described above, crosses were made between plants having the original state of a_1^{m-1} in one chromosome 3 and a_1 in the homologue, with plants having one of the following derived states: state 5718, state 5719A-1, and state 5720. ~~The types of kernels appearing on the ears these crosses produced will not be considered here in detail.~~ As ~~stated~~ mentioned earlier, plants having gametes each carrying a mutant of a_1^{m-1}. the original state of a_1^{m-1} give$ rise to many ~~germinal mutations~~. The majority of ~~kernels and plants that have one such germinal mutation in them~~ express the higher alleles of A_1 in both kernel and plant ~~exhibit the full A_1 phenotype~~. ~~However, other germinal mutations give~~ Some of them, however, express much lower ~~xxxxxx~~ ~~lesser~~ grades of pigment intensity ~~both~~ in kernel and plant and there is a wide range in the degree of these. Many alterations of a_1^{m-1} that effect this change in gene action ~~of these~~ are stable in the presence of Spm. ~~This could be observed directly~~ was unaffected, initially, by the phenotype in some kernels on ears produced ~~by the~~ from cross ~~conducted with~~ of plants carrying state 5718 or state 5719A-1 to plants carrying the original state of a_1^{m-1}. Before these phenotypes are discussed, those that appear on ears produced by cross of plants ~~considering this, attention will be given to the behavior of the original~~ having the original state of a_1^{m-1} ~~in plants that carry this state in one chromosome 3 and a_1 in the homologue.~~ by plants homozygous for a_1 will be described

In the summer of 1951, 48 plants were grown from variegated kernels appearing on ears produced by cross of the original a_1^{m-1} carrying plant to plants that were homozygous for a_1. These kernels exhibited a pattern of variegation that was common to most of the variegated kernels produced by

test crosses conducted with the orignal a_1^{m-1} carrying plant (phots). The plants derived from these kernels likewise exhibited the same bizarre types of variegation pattern that charact rized the appearance of the kernels. It was obvious that change of a_1^{m-1} was occurring in a number of cells of the plant and many of them occurred early in plant development. The plants were composites of distinct sectors in each of which a particular phenotype was expressed. Many of them exhibited the A_1 phenot pe, In others, the pigment was uniformly distributed withi the secotr but the intensity of it was low. Still other sectors were distinguished by distinctive types of variegation patterns within them. These were expressed as streaks of the A_1 type pigment in a non-pigmented background, and the number and size of these streaks was not the same in all sectors exhibiting variegations patterns. In a few plants, the type of pattern of variegation exhibited by one tiller differed greatly from that of the main stalk and of other tillers of ths same plant.

Some of the variegated plants that were a_1^{m-1}/a_1 in constitution were used as female parents in crosses with plants homozygous for a_1 and sh_2. The types of kernels appearing on the resulting ears are given in A of table 17. Pollen of two plants were used on silks of plants carrying

a modified state of a_1^{m-1} (state 5718 or 5719A-1). To serve as a control ~~to such crosses~~, part of the pollen collected from each plant was placed on silks of ears of plants that were homozygous for a_1. The kernel types on ~~these~~ ears are shown in B of table 17. In this table, the kernels ~~that appeared to be fully~~ A_1 ~~in phenotype~~ are placed under the heading "A_1". Under the heading of "pale" are placed those kernels that were uniformly pigmented but in which the intensity of ~~this~~ ~~was~~ lower ~~than that produced by~~ A_1. Within this class, ~~there was a range in~~ intensity from very faintly ~~pigmented~~ ~~in~~ some ~~kernels~~ to medium dark ~~for~~ others. All kernels exhibiting variegation for anthocyanin pigmentation are ~~entered~~ under the heading "variegated". Most of these kernels ~~exhibited~~ had both large ~~and~~ small areas with the A_1 phenotype ~~and~~ pale areas. In the last column of ~~this~~ table 17 are entered the number of kernels ~~expressing~~ a markedly altered pattern of variegation, similiar in types to those ~~that give rise to the~~ altered states that have been considered in previous sections. It may be seen that the number of them is low. However, other changed states, less easy to detect, were being produced. One of them was detected because it was present in the cell whose descendent cells gave rise to an ear ~~that~~ had been used in a cross with a plant homozygous for a_1 and sh_2.

The pattern expressed by the variegated kernels on the resulting ear was of one type and it differed from that given by the original state of a_1^{m-1}. This similarity in pattern made it possible to know that an altered state was present in the cells that gave rise to this ear. In the presence of Spm, this altered state produces many small A_1 dots, located very close to each other, and also some larger areas exhibiting the A_1 phenotype (Photo.). To the naked eye, some kernels having this pattern of variegation may appear to be uniformly pigmented. However, microscopic examination of them reveals the pattern of A_1 spots that is responsible for this deception. The dots of A_1 are so close together that there distinctiveness can not be seen except when viewed under magnification. (In all studies of variegation, kernel types on ears are examined under magnification in order that details of importance in them would not be undetected.) In the absence of Spm, kernels in which this state of a_1^{m-1} is present have pigment in them, but this is so light in intensity that detection of it sometimes is difficult. The types of kernels on the ear of the original plant having this state 5996-4 are given in C of table 17. In plant 5996-4, one Spm was present and it was carried in one of its two chromosomes 6. (See footnote to table 17.)

On the ears produced by crosses of plants that were a_1^{m-1} (original state)/ a_1 to plants that carried either state 5718 or 5719A-1/a_1, many of the kernels that received the original state of /a_1^{m-1}, or a mutant derived from it, could be recognized. Likewise, those that received a_1 from the plant having the original state of a_1^{m-1} and the derived state of a_1^{m-1} from the other parent also could be recognized. This may be seen from the photograph of kernel types appearing on one such ear (photo.). However, kernels with a new phenotype appeared on these ears. They had the pattern of A_1 dots given by the derived state of a_1^{m-1} but these appeared on a pale/pigmented background instead of a colorless background. This suggested that the plant carrying the original state of a_1^{m-1} had contributed a mutant of a_1^{m-1} giving a reduced grade of pigment intensity and that this mutant was stabe in the presence of Spm. Tests of plants derived from such kernels indicated the correctness of this interpretation. In other words, the original state of a_1^{m-1} was giving rise to mutants that were stable in the presence of Spm.

Some of the pale mutants produced by the original state of a_1^{m-1} that are stable /in the presence of Spm very much resemble in their phenotypic expression that given by some of the derived states in the absence of Spm. This is strikingly illustrated by one type that resembles that produced by state

5719A-1. In the absence of Spm, kernels having state 5719A-1 are deeply pigmented whenever Pr is present in them. However, when the kernels are homozygous for the recessive allele, pr, the kernels are only lightly pigmented. Some of the stable mutants derived from the original state of a_1^{m-1} give these same phenotypes with the alleles of Pr. It would appear that the production of either a stable mutant or a new state by the original state of a_1^{m-1} is associated with the fate of the controlling element at the a_1^{m-1} locus. Its removal or total inactivation could give rise to the stable mutants whereas a slight shift in its location, without inactivation, may be responsible for the origin of some of the altered states. It is apparent, nevertheless, that the production of stable mutants by the original state of a_1^{m-1} is far more frequent than the production of obviously altered states of the types that have been described in this report.

In the cross of a_1^{m-1}(original state)/ a_1 by a_1^{m-1} (state 5718 or 5719A-1)/ a_1, the Spm number was known only in the plant carrying the derived state. The Spm number in most plants having the original state could not be determined readily from the types of kernels appearing on ears produced by testcrosses conducted with each. The frequency of occurrence of germinal mutation was too high. Nevertheless,

the types of kernels appearing on the ears produced by/the cross of plants carrying the original state to plants carrying the derived states, whose constitutions were given above, is instructive, and the types of kernels appearing on six ears produced by this type of cross will be given. There was a total of 2175 kernels with the following phenotypes: 277 with/the A_1 phenotype, 71 that were uniformly pale colored and had a derivative of the some of these obviously the original state of a_1^{m-1} and introduced by the parent having it. Eighteen other kernels were uniformly pale colored but supperimosed on this were dots or spots of A_1 with a pattern given by the derived state of a_1^{m-1} used in the cross. There were 1207 variegated kernels that fell into two distinct classes: 602 exhibited the pattern that is characteristically produced by the original state of a_1^{m-1} and in some of these, the presence of the derived state was suspected, and 605 that exhibited the dots or spots of A_1/characteristically produced by the derived state and in what appeared to be a colorless background. In addition, there were 602 colorless kernels. Tests of plants derived from kernels exhibiting the variegated pattern of the derived state on a pale pigmented background, and those derived from ones in which both the original state and the derived state were suspected to be preset, confirmed the suspected constitutions of the kernels from which each was derived. From the former,

the presence of a stable mutant in one chromosome 3 and the derived state either 5718 or 5719A-1, as the case may be, in the other chromosome 3. Plants derived from the latter type of kernel had the original state of a_1^{m-1} in one chromosome 3 and the derived state in the other.

Tests of a_1^{m-1} behavior that have been discussed in detail in previous sections of this report were all conducted during the early period of study of a_1^{m-1}. From them was developed an interpretation of the primary mode of operation of the system responsible for control of gene expression at a_1^{m-1}. The origin and behavior of different states of a_1^{m-1} were considered, and the discovery of an independently located element, Spm, whose mode of action was outlined, was likewise treated. Nevertheless, up to this time in the study of a_1^{m-1}, no previse tests of this interpretation had been conducted.

The interpretation that had been developed implied the following conditions. Spm is ~~xx xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx~~ is the independently located element in the a_1^{m-1} system, and it is subject to transposition from one location to another in the chromosome complement. In its presence, change occurs at the a_1^{m-1} locus and this may lead to one of two main consequences. One of them results in a stable mutant expression; each such mutant expresses one mode of gene action, recognized by the ~~degree and~~

and kind of anthocyanin pigment that ~~it produces~~ in plant and kernel. The second ~~results~~ in an altered state of a_1^{m-1}, each state expression thereafter one particular type of presence to Spm. This is related to control by the altered a_1^{m-1} itself of the time during development when subsequent change will occur to it, the types of mutants that these latter changes will induce, and the number of cells in which such events will occur. The number of Spm elements present in the nuclei of a plant or kernel does not alter these qualities of a particular state of a_1^{m-1}. In the absence of Spm, most states of a_1^{m-1} functions in the production of anthocyanin pigment in both plant and kernel, and again, the type of pigment and level of its production is a quality of state of a_1^{m-1}. This function is suppressed when Spm is present in the nuclei. Removal of Spm from the nucleus of a somatic cell will allow the potentially functional activity of the state of a_1^{m-1} that is present to be expressed in its descendent cells.

The above statements incorporate the primary conditions imposed by the interpretation. ~~More~~ evidence ~~was requited in order to test~~ the general validity of this interpretation. Therefore, such test were conducted during the summer of 1954. There were many ~~such tests~~ and they will be the subject of the following section of this report ~~that follows~~.

~~Establishment of the Validity~~ Verification of the interpretation of mode of operation of the Spm element and of the individuality of state of a_1^{m-1}

The interpretation of mode of control by Spm of gene expression at a_1^{m-1}, outlined in previous sections, required precise verification, and tests devised for this purpose were conducted during the summer of 1954. The interpretation considers that no Spm is present in the uniformly pale ~~colored~~ pigmented kernels on ears derived from crosses entered in tables 2 to 8 and 10 to 15, but that it is present in all kernels exhibiting deeply pigmented areas in a colorless background. It also considers that each state of a_1^{m-1} will respond to any one isolate of Spm in its own manner, ~~that is characteristic of the state. In other words, it will be the state of a_1^{m-1} that will govern the pattern of variegation when any one Spm is present.~~ To illustrate, if pollen of a plant homozygous for a_1 and carrying 1 Spm is placed on the silks of corn of a number of plants, each derived from a pale colored kernel and in each of which a different state of a_1^{m-1} is present, then on the ear produced by each plant, one-half of the a_1^{m-1} carrying kernels should be pale colored and half should be variegated. On each ear, the pattern of variegation among the variegated kernels should express the intensity of this depending on the state that is present, among the variations one particular uniform pattern of variegation characteristic of the state of a_1^{m-1} present in the ear bearing plant, and it should be that controlled by the particular state of a_1^{m-1} present in the ear bearing plant.

If the pollen parent should have 2 ~~xxxxxxxxxxxx~~ non-linked Spm elements,

then on each of the resulting ears there should be a ratio of one pale colored kernel to 3 variegated kernels among the a_1^{m-1} carrying kernels and again, the pattern exhibited by them should be that fixed by the state of a_1^{m-1} that is present.

Several types of test-cross were devised that made it possible to learn whehter or not Spm were present in any one plant, and to demonstrate with certainty the control of phenotype by state of a_1^{m-1}, both in the presence and absence of Spm. These were conducted during the summer of 1954. The plants to be tested were derived from selected kernels on ears produced by crosses made in previous growing seasons. The types of kernels selected, the number of plants derived from each type, and the origin of the ear from which each selection was made, is given in table 18. All kernels derived from one ear were grown under one culture number, that entered in the last column of this table. However, the kernels of each class were separated from one another, and grown under the same culture number but to which was added a distinctive letter of the alphabet. If other genetic markers also were segregating among the kernels on the ear, all these having one phenotype were sown separately from those with another, each given the same culture number but appropriately distinguished by a letter of the alphebet following the culture number.

The most critical types of test-cross were conducted with plants belonging to three different categories. The plants belonging to the first of these were uniformly pigmented. They were derived from pale colored kernels on the self-pollinated ears of plants having one particular state of a_1^{m-1}. Each of the tester plants in this first category was homozygous for one particular state of a_1^{m-1} that was present in the parent plant. They were also homozygous for Sh_2. Within this category, four different states were represented, and these are given in part I of table 19. The culture number of each may be traced through table 18, as well as their constitutions with regard to genetic markers carried in chromosomes 5, 6, and 9, are also given in this table.

If the plants in part I of table 19 are used in crosses of the following types, only pale colored, Sh_2 kernels should appear on the resulting ears:

(1) Crossed with plants of the standard a_1/a_1 tester stock.

(2) Self-pollinated or sib-crossed

(3) Intercrossed with plants in the same category, bringing together two different states of a^{m-1}.

(4) Crossed to plants derived from the pale class of kernels in table 18.

(5) Crossed to some of the plants derived from the colorless, sh_2 cloud cultures entered in kernels in/table 18.

If the plants in part I of table 19 are used in making crosses of the following type, both pale colored, Sh_2 kernels and variegated, Sh_2 kernels should appear on the resulting ears:

(1) Crossed to some of the plants derived from the colorless, sh_2 cloud kernels in cultures entered in table 18.

(2) Crossed to plants derived from the variegated class of kernels in cultures of table 18 in which the constitution of the plants are a_1^{m-1} Sh_2/ a_1 sh_2.

From cross (1) immediately above, all the variegated kernels on the resulting ear should exhibit a pattern that conforms with that expected to be produced by the state of a_1^{m-1} present in the tester plant. If one ear of the a_1/a_1 plant received pollen from a tester plant homozygous for one state of a_1^{m-1} and another ear of the same plant received pollen from a tester plant that was homozygous for another state of a_1^{m-1}, then among the variegated kernels on each ear, a distinctive pattern of variegation could be expressed, one pattern among the variegated kernels on one ear and another pattern among those on the other ear. The difference in pattern on the 2 ears would reflect the individual type of response of each state of a_1^{m-1}, contributed by the tester stock, to the presence of the same Spm element.

From type-cross (2) immediately above, two class of variegated kernels should appear on the resulting ear. One should exhibit the variegated pattern produced by one state of a_1^{m-1} superimposed on that produced by the other. These would be the kernels receiving a_1^{m-1} from each parent. The second class should exhibit only the pattern that is produced by the state of a_1^{m-1} delivered to it by the tester plant, and these kernels would be those that received a_1 from the plant being tested, and a a^{m-1} from the tester plant.

On the ears produced by the type-crosses described above, it should be possible to determine the Spm number in that part of the plant that produced the ear used in the cross. This could be deduced from the ratio of pale to variegated kernels that appears on the ear. Also, if the plant being tested were heterozygous for one or more of the genetic markers in chromosomes 5, 6, and 9, given in table 19, then the location of Spm with respect to these markers could be determined, provided that the tester plant was homozygous for the recessive alleles at these markers.

More than 500 of the cultures entered in part I of table 19. The described test-crosses were made using plants in the first category, those From the kernel types on the resulting ears it was possible to determine not only the presence or absence of Spm in many plants, whose phenotypes were known, and to correlate this with the phenotype of the plant, either variegated or uniformly pigmented

~~It was~~ possible to determine ~~but~~ also, ~~when present~~, the number of Spm elements ~~that~~ were present in any one tested part of a plant. In many plants ~~it was also possible to determine~~ the location of Spm with reference to the three given genetic markers, Pr in chromosome 5, Y in chromosome 6 and Wx in chromosome 9. could be determined These tests also ~~served to~~ confirm the individuality of the different states of a_1^{m-1} in the modes of control of pattern of variegation in the presence of Spm, and also the type of gene expression of the above states are given in its absence. Before evidence ~~of this is~~ presented, the usefulness of tester plants in each of the two remaining categories will be described.

For some types of test, it would have been desirable to use plants that were homozygous for both a_1^{m-1} and sh_2. At the time, no plants of this constitution had been constructed. Therefore, kernels that were a_1^{m-1} sh_2/ a_1 sh_2 appearing on ears produced by the crosses entered in tables 5 and 8 were selected and plants grown from them. All of them had the ~~xxxx~~ 5719A-1 state of a_1^{m-1}. Four plants were derived from pale colored, sh_2 kernels, and 6 plants were derived from variegated, sh_2 kernels. The ears from which each of these were selected is indicated in table 18. and to what extent the many [illegible] The ~~phenotypes~~ constitutes of these plants with respect to Spm and the alleles of Pr, Y, and Wx are entered in parts II and III of table 19.

The plants in Part II of table 19 were all uniformly pigmented. When pollen from any one of them was placed on silks of ears of plants in the standard a_1 tester stocks, one-half of the kernels on the resulting ears were uniformly pale colored, sh_2, and the other half were colorless, sh_2. When the same pollen was placed on silks of ears produced by plants derived from the pale, Sh_2 class of kernels which had the constitution a_1^{m-1} Sh_2 / a_1 sh_2 (see table 18) the ratio of kernel types on the resulting ears were as follows: 2 pale colored, Sh_2 : 1 pale colored, sh_2 : 1 colorless, sh_2. However, when the pollen was placed on silks of ears of sister plants, derived from the variegated kernels, pale and variegated kernels appeared on the resulting ear, not only in the Sh_2 class but also in the a_1^{m-1} / sh_2 class carrying This indicated that the variegated plant carried an Spm to which the a_1^{m-1} in the a_1^{m-1} / sh_2 chromosome derived from the tester stock could respond. It was important to compare the ratio of pale to variegated in the Sh_2 class with that in the a_1^{m-1} carrying sh_2 class in order to learn whether or not Spm was located in the same chromosome 3 that had a_1^{m-1} and Sh_2. If so, the ratio of variegated to pale kernels in the Sh_2 class would be greater than in the sh_2 class. Tests conducted during the summer of 1954 did not reveal a case of this but similiar types of test conducted in later years did reveal the presence of an Spm element in chromosome 3 in some plants.